

13. A. Y. C. Huang *et al.*, unpublished data. B78H1-1P produced a small but measurable titer of transmissible helper virus. Thus, in the case of B78H1-1P, *in vivo* virus transmission cannot be ruled out as a potential mechanism for NP presentation on host-derived MHC class I molecules. In contrast to B78H1-1P, CT26-1P did not produce any transmissible helper virus; thus, *in vivo* virus transmission does not account for the results shown in Fig. 3.
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16. Naive C57BL/6 (*H-2<sup>b</sup>*) → BALB/c × B6 (*H-2<sup>dmb</sup>*) female chimeric mice were injected intraperitoneally (IP) with  $1.5 \times 10^7$  live DBA/2 splenocytes from a female mouse in 0.1 ml of 1× Hanks' balanced salt solution. As controls, naive female BALB/c (*H-2<sup>d</sup>*) mice, BALB/c (*H-2<sup>d</sup>*) → BALB/c × B6 (*H-2<sup>dmb</sup>*) chimeric mice, and DBA/2 mice were also injected IP with the same number of live DBA/2 splenocytes. Two mice were used in each group in one single experiment. Two weeks after immunization, splenocytes from immunized mice were stimulated, and P815 (derived from DBA/2 mice) targets were labeled with  $^{51}\text{Cr}$  and added to the effector cells at a concentration of 3000 cells/well.
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# Cloning of a T Cell Growth Factor That Interacts with the $\beta$ Chain of the Interleukin-2 Receptor

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A cytokine was identified that stimulated the proliferation of T lymphocytes, and a complementary DNA clone encoding this new T cell growth factor was isolated. The cytokine, designated interleukin-15 (IL-15), is produced by a wide variety of cells and tissues and shares many biological properties with IL-2. Monoclonal antibodies to the  $\beta$  chain of the IL-2 receptor inhibited the biological activity of IL-15, and IL-15 competed for binding with IL-2, indicating that IL-15 uses components of the IL-2 receptor.

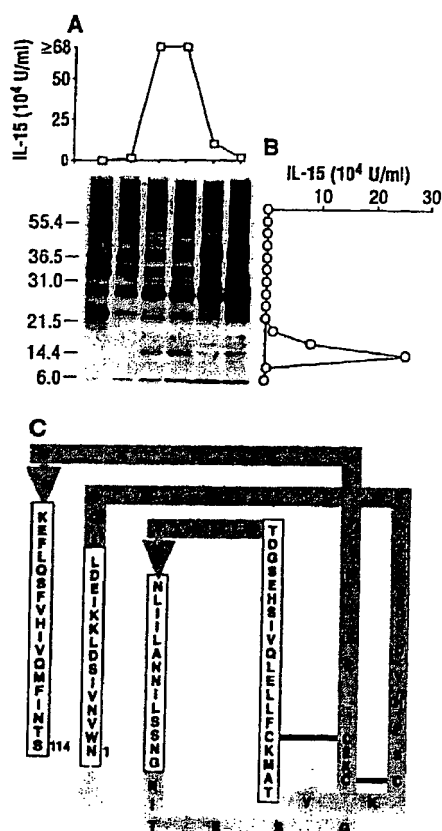
The proliferation and differentiation of T lymphocytes is regulated by cytokines that act in combination with signals induced by the engagement of the T cell antigen receptor. A principal cytokine used by T cells during immune responses is IL-2 (1), itself a product of activated T cells. IL-2 also stimulates a number of other cell types, including B cells, monocytes, lymphokine-activated killer cells, natural killer cells, and glioma cells (2). IL-2 interacts with a specific cell surface receptor (IL-2R) that contains at least three subunits,  $\alpha$ ,  $\beta$ , and  $\gamma$  (3). A number of other cytokines also stimulate the proliferation of T cells, and recent evidence has suggested that the receptors for several of these cytokines include the  $\gamma$  chain of IL-2R (4). We describe a cytokine whose biological activity resembles that of IL-2 and which also uses components of IL-2R.

In the course of testing supernatants from a simian kidney epithelial cell line, CV-1/EBNA (5), for cytokine activity, it was discovered that these cells produced a soluble factor capable of supporting proliferation of the IL-2-dependent cell line, CTLL (6). The protein responsible for this biological activity was purified from serum-free supernatants of CV-1/EBNA cells by a combination of hydrophobic interaction and anion-exchange chromatography, high-pressure liquid chromatography

(HPLC), and SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1A, bottom). Analysis of the biological activity in the final HPLC fractions (Fig. 1A, top) and in horizontal slices of the SDS-PAGE gel (Fig. 1B) indicated that the activity coincided with a band of approximately 14 to 15 kD. This protein was blotted to a polyvinylidene difluoride (PVDF) membrane, and the NH<sub>2</sub>-terminal residues were directly sequenced. We used degenerate oligonucleotide primers on the basis of this amino acid sequence and the polymerase chain reaction (PCR) to clone a 92-base pair (bp) complementary DNA (cDNA) fragment from mRNA of CV-1/EBNA cells. This cloned cDNA fragment was used to probe a plasmid library containing cDNA inserts prepared from mRNA of CV-1/EBNA cells. A full-length cDNA clone was obtained that encodes a 162-amino acid precursor polypeptide containing an unusually long 48-amino acid leader sequence that is cleaved at the experimentally determined NH<sub>2</sub>-terminus to form the mature protein. The amino acid sequence (Fig. 1C) exhibits no similarity to any protein in GenBank or EMBL databases. However, IL-15 and IL-2 sequences were compared to determine if there might be structural similarities. The three-dimensional (3D) structure of IL-2 (7) consists of a four-helix bundle, and IL-2 belongs to the helical cytokine family (8). Although the members of this family show no sequence similarity, they show many structural similarities, and IL-15 is no exception. The secondary struc-

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ture prediction for IL-15 shows strong helical moment for regions 1 to 17 and 94 to 112 and supports a four-helix bundle-like structure for this protein. We used the IL-2 structure as a template to build a 3D model of IL-15 with FOLDER, a distance geometry-based homology modeling package (9). The model suggests two disulfide cross-links one of which,



**Fig. 1.** Purification and cloning of IL-15. (A) (lower panel) Silver-stained gel after SDS-PAGE of active fractions from the final HPLC of the IL-15 purification (17). Molecular sizes are indicated on the left (in kilodaltons). (Upper panel) The activity of each HPLC fraction is graphed directly above the corresponding lane of the gel. (B) The activity eluted from each horizontal slice (of a similar gel of the peak fraction) is depicted alongside the corresponding position on the gel. (C) The deduced amino acid sequence of mature simian IL-15 (18) in a schematic representing its predicted folding topology, with four helices (boxed sequences) in an up-up-down-down configuration, three loops connecting the helices (shaded sequences), and two disulfide crosslinks (darker shading), as suggested by homology modeling of IL-15 with the crystal structure of IL-2 as template with use of the FOLDER program (9). The sequence of simian IL-15 has been submitted to GenBank-EMBL (accession number U03099). Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

Cys<sup>42</sup>-Cys<sup>88</sup>, is analogous to the only disulfide in IL-2.

Northern (RNA) blot analysis of a variety of human cell lines identified the IMTLH bone marrow stromal cell line as a source of human IL-15. The simian-derived cDNA was used to probe an IMTLH cDNA library. A single hybridizing clone was isolated that shares 97% sequence identity in the coding region with the simian IL-15 cDNA. The human IL-15 cDNA contains a 316-bp 5' noncoding region preceding an open reading frame of 486 bp and a 400-bp 3' noncoding region.

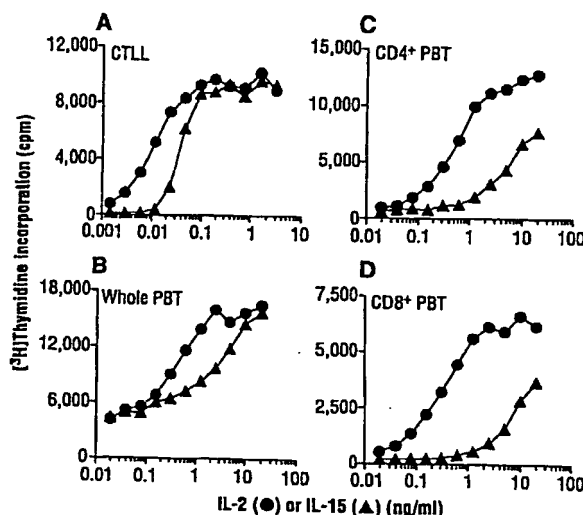
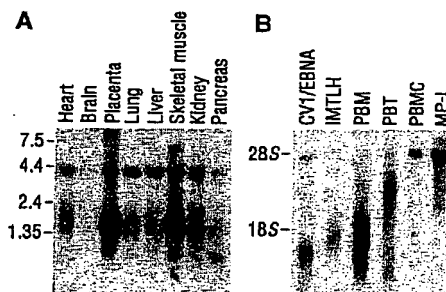
Expression of IL-15 mRNA was detected by Northern blot analysis of several human tissues. IL-15 mRNA was most abundant in placenta and skeletal muscle (Fig. 2A), with detectable levels in heart, lung, liver, and kidney. The best sources of IL-15 mRNA so far observed have been adherent peripheral blood mononuclear cells [monocyte enriched (PBMCs)] and epithelial and fibroblast cell lines such as CV-1/EBNA and IMTLH. Freshly isolated, uncultured PBMCs (Fig. 2B) also express very low levels of IL-15 mRNA. Activated peripheral blood T cells (PBTs), a rich source of

IL-2 and IFN- $\gamma$  mRNA, express no detectable IL-15 mRNA, nor do B lymphoblastoid cell lines such as MP-1.

Simian IL-15 was expressed in yeast from a cDNA in which the 5' untranslated sequences and the 48-amino acid leader sequence had been removed. Purified recombinant simian IL-15 stimulated the proliferation of CTLL cells (Fig. 3A) and also phytohemagglutinin (PHA)-activated PBTs (Fig. 3B), in each case to the same extent and with similar potency as IL-2. Both the CD4<sup>+</sup> and CD8<sup>+</sup> subsets of PBTs responded to IL-15 after activation with PHA (Fig. 3, C and D). IL-15 was also found to stimulate the proliferation of murine antigen-specific T cell clones, including helper and cytotoxic clones (10).

In addition to stimulating the proliferation of CTLL cells and PBTs, IL-15, like IL-2, induces the generation of cytolytic effector cells in vitro (Fig. 4A). The primary in vitro induction of alloantigen-specific cytotoxic T lymphocytes (CTLs) was measured in mixed leukocyte cultures. Non-antigen-specific lymphokine-activated killer (LAK) cells were generated in syngeneic cultures of PBMCs. IL-15 was at

**Fig. 2.** Northern analysis of human cells and tissues for IL-15 mRNA. Northern blots containing polyadenylated RNAs were hybridized with an antisense probe made by transcription of a human IL-15 cDNA (19). (A) Human tissue blot (Clontech, Palo Alto, California). Molecular sizes are indicated on the left (in kilobases). (B) Blot containing RNA from CV-1/EBNA cells (simian) and human cells including IMTLH cells, a cell line derived from human bone marrow stromal cell culture transformed with pSV3Neo; PBM, adherent peripheral blood mononuclear cells greatly enriched for monocytes, cultured for 4 hours in lipopolysaccharide; PBT (E-rosetted) stimulated for 4 hours with ionomycin and PMA; PBMC, freshly isolated uncultured peripheral blood mononuclear cells; and MP-1, an EBV-transformed B lymphoblastoid cell line. Cross-hybridization of the IL-15 probe is also seen to 28S ribosomal RNA.



**Fig. 3.** Regulation of proliferation by IL-15. Purified recombinant simian IL-15 (20) was compared to human recombinant IL-2 (Cetus Oncology Corporation, Emeryville, California), at various concentrations, for induction of proliferation of murine CTLL cells (A) or of PHA-activated human peripheral blood T lymphocytes and their derivative CD4<sup>+</sup> and CD8<sup>+</sup> subsets (B through D) (15).

least as potent and effective as IL-2 in these assays.

The similar biological properties of IL-15 and IL-2 suggested that IL-15 might function by inducing the production of IL-2 or by using the same receptor. A neutralizing antibody to IL-2 did not inhibit the induction of LAK cells by IL-15 (Fig. 4B), indicating that IL-15 probably does not function in this assay by inducing IL-2. Furthermore, an antibody to IL-2R $\alpha$  (anti-IL-2R $\alpha$ ) (2A3) failed to inhibit IL-15-induced proliferation of PHA-activated

PBMCs (PHA-PBMCs), although this antibody inhibited IL-2-induced proliferation (Fig. 4C). This is further evidence that IL-15 can function in the absence of IL-2. The antibody to IL-2 did, however, inhibit induction of CTLs by IL-15 from resting PBMCs, indicating that under these conditions IL-15 either induces IL-2 or synergizes with the low level of endogenously produced IL-2 to induce functional T cells.

To further compare IL-2 and IL-15, we examined the binding of radiolabeled IL-2 and IL-15 to PHA-activated PBMCs (Fig.

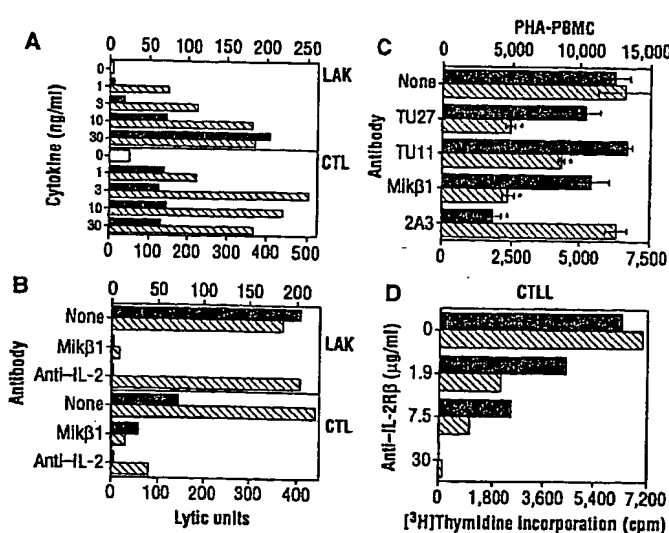
5). Scatchard analysis indicated that IL-2 binding to these cells shows the well-known two classes of high- (dissociation constant ( $K_d$ ) =  $1.48 \times 10^{-11}$  M) and low-affinity receptors, but IL-15 appears to have only a single high-affinity [ $K_d$  =  $2.5 \times 10^{-11}$  M] binding site. The number of IL-15 receptors per cell is very similar to the number of high-affinity IL-2 receptors (573 and 426 sites per cell, respectively). To test whether subunits of IL-2R might participate in IL-15 binding, we measured the ability of IL-15 to inhibit binding of radiolabeled IL-2 to receptors on the human YT cell line (11). The YT cell line was chosen because of the low expression of IL-2R $\alpha$  on these cells. IL-15 very effectively competes with IL-2 for binding to YT cells (Fig. 6), suggesting that IL-2 and IL-15 share common binding sites on these cells. IL-1 and IL-4, both of which bind to their own sites on YT cells, failed to compete for IL-2 binding. Experiments with blocking monoclonal antibodies confirmed the participation of IL-2R chains in IL-15 binding and function. Whereas the anti-IL-2R $\alpha$  did not inhibit IL-15-induced proliferation of T cells, antibodies to IL-2R $\beta$  inhibited all activities of IL-15, including the generation of LAK cells and CTLs (Fig. 4B) and the proliferation of PHA-PBMCs (Fig. 4C) and CTLL cells (Fig. 4D). Antibodies to IL-2R $\beta$ , such as TU27, have been shown to neutralize IL-2-dependent T cell proliferation (such as in Fig. 4C) only when combined with anti-IL-2R $\alpha$  (12). These antibodies to IL-2R $\beta$  consistently inhibit IL-15 more effectively than they inhibit IL-2. In fact, even antibodies like TU11, which do not inhibit IL-2 under any circumstances (13), will inhibit IL-15, suggesting that IL-15 binds to IL-2R $\beta$  differently than does IL-2. These data confirm that stimulation by IL-15 requires interaction of IL-15 with components of IL-2R, including IL-2R $\beta$  and probably IL-2R $\gamma$ , but not IL-2R $\alpha$ .

Although some activities are shared by IL-2 and IL-15, there are also differences between the biological effects of the two cytokines. For example, the IL-3-dependent cell line, 32D (14), expresses the complete IL-2R and proliferates vigorously in response to IL-2, but responded poorly or not at all to IL-15 (Fig. 7). These data suggest that IL-15 may use another receptor component not shared by IL-2R and not expressed by 32D cells. Thus, there are likely to be biological activities of IL-15 not shared by IL-2.

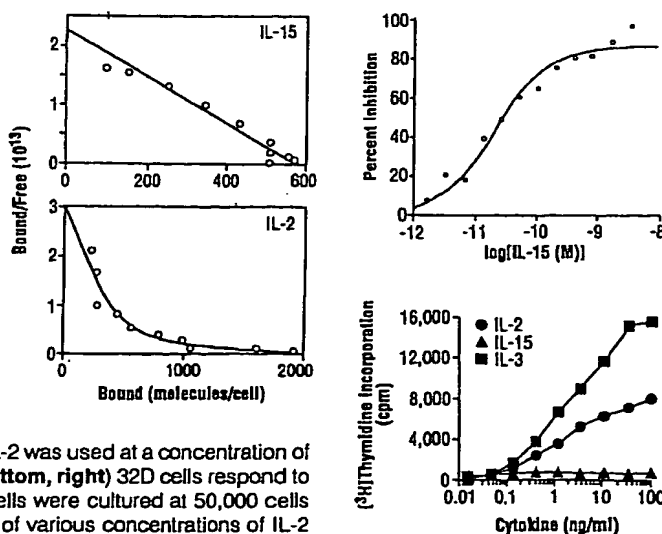
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**Fig. 4.** Inhibition of IL-15 biological activities by antibodies to IL-2R $\beta$ . (A) Cytolytic activity (lytic units) from human mixed leukocyte cultures (CTL) or syngeneic cultures (LAK) containing various concentrations of cytokine was measured (21) against either the specific allogeneic target cell (CTL) or against the Daudi lymphoblastoid cell line (LAK). (B) Cytolytic activity from cultures parallel to those in (A) containing Mik $\beta$ 1 anti-IL-2R $\beta$  (10  $\mu$ g/ml) or sheep anti-IL-2 (100  $\mu$ g/ml) in the presence of either IL-2 or IL-15 (10 ng/ml). (C) Proliferation of PHA-stimulated PBMCs (16) recultured with the indicated antibodies to IL-2R $\beta$  (each at 10  $\mu$ g/ml), Mik $\beta$ 1, TU27, and TU11 (22), or to IL-2R $\alpha$ , 2A3 (22), in the presence of IL-2 (top y axis) or IL-15 (bottom y axis) (each at 3 ng/ml). \*The mean is different than "none" at the 0.05 level according to Dunnett's procedure for comparing several treatments with a control (23). The data represent the means of four replicate cultures, and the experiment shown is representative of at least five experiments using these and other antibodies to IL-2R $\beta$ . (D) Proliferation of CTLL cells cultured with various concentrations of anti-IL-2R $\beta$  (Pharmingen, San Diego, California) in the presence of IL-2 or IL-15 (each at 0.1 ng/ml). The response of the CTLL cells to IL-4 was unaffected by this antibody. Shaded bars, IL-2; hatched bars, IL-15.



**Fig. 5.** (left) Comparison of  $^{125}$ I-IL-15 and  $^{125}$ I-IL-2 binding to PHA-activated PBMCs (24). Scatchard representation of the binding data. **Fig. 6.** (top, right) Inhibition of  $^{125}$ I-IL-2 binding to YT cells by IL-15. Binding of radiolabeled IL-2 to YT cells (25) was carried out in the presence of increasing concentrations of unlabeled IL-15. Cells were first incubated with the 2A3 antibody to IL-2R $\alpha$  for 60 min, 4°C at 40  $\mu$ g/ml.  $^{125}$ I-IL-2 was used at a concentration of  $5 \times 10^{-10}$  M.



**Fig. 7.** (bottom, right) 32D cells respond to IL-2 but not to IL-15. 32D cells were cultured at 50,000 cells per culture in the presence of various concentrations of IL-2 or IL-15.

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15. PBTs were selected from fresh PBMCs by E-rosette formation, and CD4<sup>+</sup> and CD8<sup>+</sup> subsets were further isolated by antibody affinity to paramagnetic microspheres with magnetic cell sorter (MACS, Miltenyi Biotec, Sunnyvale, CA). T cells were activated for 72 hours with PHA followed by 24 hours in IL-2-containing medium. T cell blasts were then harvested, washed, and used. Test cultures contained 50,000 T cells per culture or 2000 CTL cells per culture. Culture medium was supplemented as described [K. Grabstein *et al.*, *J. Exp. Med.* 163, 1405 (1986)], and 0.5  $\mu$ Ci of [<sup>3</sup>H]thymidine was added for the last 4 hours of culture. Cells were collected onto glass fiber filters and radioactivity was determined by avalanche gas ionization.
16. PBMCs, prepared from fresh whole blood by Ficoll Hypaque density gradient centrifugation, were activated by culture with PHA as described above for PBTs (15).
17. IL-15 was purified from 64 liters of supernatant of CV-1/EBNA cells by ultrafiltration (YM-30), hydrophobic chromatography (Phenyl Sepharose CL-4B), anion-exchange chromatography (DEAE Sephacel and Mono Q fast protein liquid chromatography), reversed-phase HPLC (C4, 5  $\mu$ m) eluted with an acetonitrile gradient in 0.1% trifluoroacetic acid (TFA) [D. L. Urdal *et al.*, *J. Chromatogr.* 296, 171 (1984)], reversed-phase HPLC eluted with an n-propanol gradient in TFA, and SDS-PAGE.
18. Proteins were electrophoretically transferred from the SDS gel to a PVDF membrane. The protein band corresponding to the IL-15 activity was cut out, and the sequence of the 33 NH<sub>2</sub>-terminal residues was determined by Edman degradation. Two degenerate oligonucleotide mixtures encoding all possible codon usages of residues 1 to 6 and the complement of all possible codon usages of residues 26 to 31 (omitting position 3 of Val<sup>31</sup>) were synthesized. First-strand cDNA synthesized from CV-1/EBNA mRNA was amplified by PCR with the oligonucleotide mixtures as primers. This yielded a 92-bp DNA fragment that was cloned into pBluescript SK<sup>-</sup>. A hybridization probe prepared from this DNA fragment was used to isolate a cDNA clone containing the complete IL-15 coding region from a cDNA library constructed from CV-1/EBNA mRNA essentially as described [D. M. Anderson *et al.*, *Cell* 63, 235 (1990)].
19. A simian IL-15 probe was prepared by labeling of the purified simian IL-15 cDNA with random primers. Northern blot analysis with this probe identified the human IMTLH bone marrow-derived stromal cell line as a source of human IL-15 mRNA. Southern (DNA) blots of pools of an IMTLH cDNA library were probed to identify a positive pool and subsequently to isolate a human IL-15 cDNA.
20. A PCR-generated DNA fragment, containing the simian IL-15 coding region minus the 48-amino acid leader sequence, was ligated into a yeast expression vector that directs secretion of the recombinant protein into the yeast medium [V. Price *et al.*, *Gene* 55, 287 (1987)]. Recombinant IL-15 was purified from the yeast supernatant as described above for the CV-1-derived IL-15 protein, excluding ultrafiltration and ion exchange. The purity and concentration of IL-15 were con-

firmed by amino acid analysis.

21. Human PBMCs from one donor ( $5 \times 10^5$  per culture) were cultured with irradiated PBMCs ( $5 \times 10^5$  per culture) from either an allogeneic donor (CTL) or from the autologous donor (LAK) in cultures containing various concentrations of either IL-2 or IL-15, or no cytokine. Cultures were done as described [M. B. Widmer *et al.*, *J. Exp. Med.* 166, 1447 (1987)] and harvested after 6 days (LAK) or 7 days (CTL) and assayed for cytolytic activity against <sup>51</sup>Cr-labeled targets. The lysis assay contained various numbers of the responding peripheral blood lymphocytes cultured with 1000 labeled targets in 200  $\mu$ l of medium in V-bottomed wells, and supernatants were collected after 4 hours of incubation. Lytic units were calculated as the inverse of the fraction of the responding culture required to generate 50% (CTL) or 30% (LAK) of the maximum specific <sup>51</sup>Cr release.
22. MikB1 was purchased from Nichirei Corp., Tokyo, Japan; TU11 and TU27 were provided by K. Sugamura, Sendai, Japan; 2A3 was produced at Immunex.
23. C. W. Dunnnett, *J. Am. Stat. Assoc.* 50, 1096 (1955).

Activated PBMCs (16) were incubated for 2 hours in medium without PHA or growth factors. IL-15 and IL-2 binding were carried out at 4°C for 60 min in RPMI 1640 containing 3% bovine serum albumin and 0.1% NaNO<sub>3</sub>. IL-2 and IL-15 were radiolabeled as described [L. Park *et al.*, *J. Biol. Chem.* 261, 4177 (1986)] and retained biological activity. Preliminary experiments established that equilibrium binding was obtained under these conditions (J. Gird and M. Ahdieh, personal communication).

25. YT cells used are a subclone of the human NK-like YT cell line and were provided by M. Caligiuri, Roswell Park Memorial Institute, Buffalo, NY.
26. We thank S. D. Lipton and R. J. Tushinski for the IMTLH cell line, M. R. Comeau and D. P. Gearing for the IMTLH cDNA library, T. Hollingsworth for DNA sequence analysis, C. J. March and M. Gerhart for protein sequence analysis, R. Jerzy for the cDNA cloning plasmid, J. King for the yeast expression construct, T. W. Tough and L. Erickson for technical assistance, and M. B. Widmer and M. K. Spriggs for reviewing the manuscript.

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## Enhancer Point Mutation Results in a Homeotic Transformation in *Drosophila*

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In *Drosophila*, the misexpression or altered activity of genes from the bithorax complex results in homeotic transformations. One of these genes, *abd-A*, normally specifies the identity of the second through fourth abdominal segments (A2 to A4). In the dominant *Hyperabdominal* mutations (*Hab*), portions of the third thoracic segment (T3) are transformed toward A2 as the result of ectopic *abd-A* expression. Sequence analysis and deoxyribonuclease I footprinting demonstrate that the misexpression of *abd-A* in two independent *Hab* mutations results from the same single base change in a binding site for the gap gene *Krüppel* protein. These results establish that the spatial limits of the homeotic genes are directly regulated by gap gene products.

The establishment of correct segmental identity in *Drosophila melanogaster* requires the proper function and expression of genes located in the antennapedia and bithorax complexes [reviewed in (1–3)]. In the bithorax complex, loss-of-function mutations typically result in transformations of posterior segments toward more anterior fates, whereas the ectopic activation of homeotic genes along the anterior-posterior axis produces dominant, gain-of-function phenotypes in which anterior segments are transformed toward more posterior identities (4–8). The initial activation of homeotic gene expression appears to be regulated by the segmen-

tation gene products (9–15). For example, mutations in the gap gene *hunchback* (*hb*) result in an anterior shift of *Ubx* expression (12), whereas mutations in *Krüppel*, *knirps*, and *giant* cause ectopic activation of *Abd-B* (13–16). In several cases, incomplete homeotic regulatory elements containing *hb* binding sites have been shown to confer spatially restricted patterns of gene expression when positioned next to a *LacZ* reporter gene (9–11). However, as a result of the large size of homeotic regulatory regions (50 to 100 kb), the precise roles of these individual elements within the context of a complete regulatory domain has remained elusive.

We have studied the *Hab-1* and *Hab-2* mutations, two gain-of-function alleles that ectopically express the *abd-A* protein (ABD-A). The *Hab-1* and *Hab-2* alleles were discovered by E. B. Lewis and I. Duncan, respectively, and Lewis has proposed that they likely affect a homeotic regulatory element (4, 15). The *Hab* mutations cause dominant transformations of portions of T3 toward A2 as a result of

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